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A STUDY OF CANINE DIROFILARIASIS IN KUALA LUMPUR

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A STUDY OF CANINE DIROFILARIASIS IN KUALA LUMPUR

By

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D.V.M.

A thesis submitted in partial fulfilment of the requirements
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June 1988

Supervisor : Dr. Rehana binti Abdullah Sani

Faculty : Veterinary Medicine and Animal Science

Although much importance has been placed on human filariasis, work on canine filariasis is limited in this country. This study was carried out to obtain a general overview on the status of canine dirofilariasis in Kuala Lumpur. Throughout a one year study, parasitological records were studied and blood samples were collected from stray dogs which were later necropsied. Blood samples were subjected to the modified Knott's concentration technique, the direct smear and the enzyme-linked immunosorbent assay which was developed using the adult worm antigen. Information was obtained from private practitioners by the use of questionaires. The period prevalence of canine dirofilariasis was 42.0 percent and 10.0 percent within the stray dog and owned dog populations respectively. The monthly prevalence indicated that dirofilarisis was prevalent throughout the year and was not

significantly influenced by rainfall and temperature changes in this area. Prevalence was significantly higher in dogs above five years of age ($p < 0.001$) and dogs of body weights above 15 kg ($p < 0.05$). Host sex and coat colour did not influence the distribution of dirofilariasis. Occult cases comprised 47 percent of the positive cases of dirofilariasis. Of these, the percentages of single-sex and dual-sex infections were 29 percent and 18 percent respectively. The range of length of the female *D. immitis* was 12.3 cm to 31.5 cm, while that for the male was 8.7 cm to 19.5 cm. The accuracies of the modified Knott's concentration technique, the direct smear and the enzyme-linked immunosorbent assay were 80.5 percent, 77.5 percent and 53.0 percent respectively. The survey of private practitioners indicates that the main adulticide, microfilaricide and prophylactic drugs currently being used are thiacetarsamide, levamisole and diethylcarbamazine. The direct smear and the filtration technique are the most frequently used diagnostic tests. Side-effects of drugs used, client's willingness to bear cost of treatment and the lack of client compliance in completing the course of medication are the main problems faced by private practitioners in the treatment of canine dirofilariasis.

Abstrak tesis yang dikemukakan kepada Senat Universiti Pertanian Malaysia bagi memenuhi sebahagian daripada syarat-syarat untuk ijazah Master Sains

KAJIAN DIROFILARIASIS ANJING DI KUALA LUMPUR

oleh

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Walaupun banyak kerja-kerja telah dijalankan ke atas filariasis manusia, kerja-kerja filariasis anjing adalah terhad di negera ini. Kajian ini telah dijalankan untuk mendapatkan pandangan menyeluruh mengenai status dirofilariasis anjing di Kuala Lumpur. Dalam masa setahun, rekod parasit dikaji dan sampel-sampel darah telah diambil dari anjing-anjing liar yang kemudiannya dibedah siasat. Teknik 'Modified Knott's concentration', smear terus dan 'enzyme-linked immunosorbent assay' telah dijalankan ke atas sampel darah. Cacing dewasa telah digunakan sebagai antigen dalam 'enzyme-linked immunosorbent assay.' Maklumat telah didapati dari klinik-klinik swasta melalui kaji selidik. Prevalen tahunan dirofilariasis anjing liar ialah 42 peratus manakala prevalen ke atas anjing bertuan ialah 10.0 peratus. Dari prevalen

bulanan, dirofilariasis adalah prevalen sepanjang tahun dan tidak dipengaruhi secara keertian oleh pertukuran hujan dan suhu di kawasan ini. Keertian prevalen adalah lebih tinggi pada anjing yang berumur lebih lima tahun ($p < 0.001$) dan anjing yang berat badan lebih dari 15 kg ($p < 0.05$). Jantina dan warna bulu tidak mempengaruhi taburan dirofilariasis. Empat puluh tujuh peratus daripada kes-kes dirofilariasis adalah kes 'occult'. Dari 47 peratus ini, 29 peratus adalah infeksi 'single-sex' dan 18 peratus adalah infeksi 'dual-sex'. Panjang purata D. immitis betina ialah 12.3 cm ke 31.5 cm, manakala panjang purata jantan adalah 8.7 cm ke 19.5 cm. Ketepatan teknik 'modified Knott's concentration' adalah 80.3 peratus, smear terus, 77.5 peratus dan 'enzyme-linked immunosorbent assay', 53.0 peratus. Kaji selidik dari klinik-klinik swasta menunjukkan ubat untuk membunuh cacing dewasa, mikrofilaria dan ubat prophylaktik yang sering digunakan ialah thiacetarsamide, levamisole dan diethylcarbamazine. Smear terus dan teknik turas adalah ujikaji diagnostik yang biasa digunakan. Kesan sampingan ubat yang digunakan, kerelaan tuanpunya anjing untuk menanggung kos perubatan yang tinggi dan kesanggupan mereka untuk menghabiskan jangkamasa perubatan adalah masalah-masalah utama yang dihadapi oleh klinik-klinik swasta dalam pengubatan dirofilariasis anjing.

CHAPTER 1

INTRODUCTION

In Malaysia, only a few species of filarial worms have been known to infect dogs. Brugia pahangi has been recorded from dogs in East Pahang (Reid and Laing, 1957), while the subperiodic Brugia malayi has been established in dogs experimentally with resulting microfilaraemia (Reid and Laing, 1959). Among all the filarial infections here, the most commonly reported canine filarial worm is Dirofilaria immitis. This is also the most pathogenic filarial worm in dogs as the infection may cause cardiopulmonary insufficiencies which can eventually result in the death of the infected animal. The prevalence rate of Dirofilaria repens has been reported to be 5.6 percent in cats in this country (Mak, 1984) and although this filarial worm is known to infect dogs, no such canine infections have been reported. Infections by other filarial worms such as Dipetalonema reconditum, D. grassi and D. dracunculoides have not been reported to occur in dogs in this country.

There have been a few reports on the prevalence rate of Dirofilaria immitis infection (Mullin, 1970; Retnasabapathy and Khoo, 1976; Kan et al., 1977; Faridah and Lee, 1981; Rajamanickam et al., 1981). However,

there is no information available on the current status of the drugs being used for prophylaxis and treatment, diagnostic tests employed and problems faced by private practitioners with regard to dirofilariasis in this country. The diagnostic tests widely used in Malaysia rely upon the detection of circulating microfilariae. Thus, cases of occult dirofilariasis, that is, heartworm infected dogs without circulating microfilariae, are a problem for diagnosis. Serological tests such as the indirect fluorescent antibody test (Ellsworth and Johnson, 1973; Wong and Suter, 1979; Dawe et al., 1980) and more recently, the enzyme-linked immunosorbent assay (Scholtens and Patton, 1983; Wagner et al., 1984; Grieve et al., 1986) have been employed and show promising results in helping to diagnose cases of occult dirofilariasis.

In view of the current situation of dirofilariasis in this country, this study was undertaken to establish the following:-

i) The period prevalence of the disease in the stray and owned canine population.

ii) The trend of the prevalence of canine dirofilariasis monthly for the period of one year.

iii) The influence of weather such as rainfall and temperature on the prevalence of the disease.

iv) Influence of host factors namely sex, age, coat colour and body weight on the prevalence of disease.

v) The length of the adult worm and its sex ratio.

vi) The sensitivity, specificity and accuracy of the direct smear and the modified Knott's concentration technique in detecting dirofilariasis.

vii) The development of the enzyme-linked immunosorbent assay as a diagnostic tool.

viii) The current status of drugs used for prophylaxis and treatment, diagnostic tests employed and problems being faced by private practitioners in Kuala Lumpur and Petaling Jaya with regard to canine dirofilariasis.

CHAPTER 2

LITERATURE REVIEW

FINAL AND INTERMEDIATE HOSTS

Dirofilaria immitis is a filarial parasite which has been reported to infect a wide range of mammalian hosts such as dogs, cats, bears, horses, orang utans and even humans. However, patent infections only occur in dogs, cats, wild felines, Californian sea lions and ferrets (Otto, 1975). Among these, only the dog acts as the main reservoir of infection as well as being the main host. Once infected, the canine host can develop polysystemic disorders with cardiopulmonary signs occuring most frequently. Congestive heart failure eventually develops, sometimes accompanied with associated organ malfunction (namely the kidney and liver), thus ultimately resulting in the death of the infected animal (Rawlings, 1981). The transmission of the infection depends solely upon the mosquito which acts as the intermediate host. This is because part of the development of the parasite occurs within the mosquito itself. Approximately 70 species (Ludlam *et al.*, 1970) of the many known species of mosquitoes in the world have been reported to allow development of *D. immitis* larvae. In Malaysia, limited information is available on the species of mosquitoes that act as the intermediate host. Cheong *et al.*,

(1981) confirmed Armigeres subalbatus to be a primary vector of D. immitis. Mansonia species have been known to harbour the infection but has been suggested to play a less important role in the transmission of D. immitis because they are uncommon in the city. A. subalbatus however, is a common urban mosquito in Malaysia which breeds in septic tanks and feeds readily on man and domestic animals such as dogs and birds (Cheong et al., 1981). In Singapore, Culex fatigans, Aedes aegypti and Aedes albopictus were found to support the development of D. immitis. These species are also found in Petaling Jaya and Kuala Lumpur and may also act as intermediate hosts here (Chellapah and Chellapah, 1968).

LIFE CYCLE

The adult worm resides in the pulmonary artery and the right ventricle of the heart. Occasionally the worms may be found in the left side of the heart, the anterior and posterior vena cavae and in ectopic sites such as the peritoneal cavity (Kelly, 1978). The viviparous female releases the unsheathed larvae, the microfilariae, which circulate in the bloodstream. These microfilariae have also been reported to cross the placenta and infect the foetus in pregnant bitches (Mantovani and Jackson, 1966). Thus, circulating microfilariae of D. immitis may be found in puppies less than six months of age. However, these microfilariae cannot develop into adult heartworms since the mosquito is required to complete the life-

cycle. Therefore the microfilariae is an end-stage unless ingested by a mosquito, and can circulate for as long as two years (Newton, 1968).

After ingestion by a female mosquito, the microfilaria undergoes two moults after which it becomes the infective third stage. This takes about 14 to 21 days under optimal temperature and humidity (Kelly, 1978). The third stage larva is deposited on the skin of the dog when the mosquito feeds. It actively migrates to a resting site in the subcutaneous, subserosal tissues, muscles or fat. Here it moults to the fourth stage larva (18 mm) long at nine to 12 days after infection and to the fifth stage larva (80 mm) long at 70 to 80 days after infection. At approximately three months after infection, the immature adult begins migrating to the right ventricle and adjacent vessels. Microfilariae are present in the uterus of the worm six months after infection by the mosquito and appears shortly in the peripheral blood (Kume and Itagaki, 1955). These adult worms have a long biotic life and infections have been reported to be patent up to seven years (Newton, 1968).

MORPHOLOGY

The adult worms are long and slender and possess a thick cuticle. The males are relatively shorter than the females and possess a distinct spiral tail. Males measure from 12 cm to 18 cm (Webber, 1955; Kelly, 1978). Soulsby (1976) reported a range of length of 12 cm to 16 cm. The female which is

viviparous, has the vulva situated at the anterior end just caudal to the oesophagus. Female worms measure between 25 cm to 30 cm (Soulsby, 1976; Kelly, 1978), while Webber (1955) reported lengths of 15.1 cm to 30.0 cm.

The microfilariae found in circulation measure 290μ to 340μ (Kelly, 1978). Mak et al. (1980) reported an average length of 234.8μ and a width of 8μ. The microfilaria of D. immitis can be differentiated from that of Dipetalonema reconditum as the former has a tapered head and a straight tail, while the latter has a blunt anterior end and a button hooked tail (Lindsey, 1965). However, these characteristics are not always easily recognised and frequently a misdiagnosis can be made. More recently, these species may be differentiated by demonstrating the acid phosphatase activity via the precipitation of a pink dye (Chaulifaux and Hunt, 1971). The D. immitis microfilaria will take the pink dye at the anterior excretory pore and anus while the entire microfilaria of D. reconditum stains pink.

PREVALENCE

A wide range of prevalence rates for dirofilariasis has been reported in Malaysia. Mullin (1970) reported a prevalence of 30.4 percent. In his study, 101 stray dogs from Kuala Lumpur and Petaling Jaya were screened for circulating microfilariae using the thick blood film technique. Necropsy revealed that 28 dogs had D. immitis infection, while the thick blood film

technique detected only 22 of these infected dogs. In another study, necropsy upon 764 stray dogs from Kuala Lumpur and Petaling Jaya over a six year period, revealed a prevalence of 25.8 percent (Retnasabapathy and Khoo, 1976). The study also revealed that there was an increasing trend in the prevalence of D. immitis infection from 1968 to 1972. Following this, in 1973 and 1974, the prevalence rates dropped by almost eight percent and 10 percent respectively compared to the figure in 1972 (36.6 percent). They attributed this to be due to the destruction of a large number of stray dogs in response to a case of rabies in March 1973. Kan et al. (1977) reported a prevalence rate of 32.4 percent. In his study, a total of 340 pet dogs in and around Seremban were screened using the thick blood film technique, the chamber method and the millipore filter method. Faridah and Lee (1981) conducted a prevalence study of dirofilariasis in pet dogs which were brought to the Small Animal Clinic of the Faculty of Veterinary Medicine and Animal Science, Universiti Pertanian Malaysia. The direct smear and modified Knott's concentration technique were employed in the screening of dogs for the presence of circulating microfilariae. Of 430 dogs screened, 18.1 percent were found to be positive for circulating microfilariae. This figure is comparatively lower than those reported by other workers (Mullin, 1970; Kan et al., 1977) and it was suggested that this was due to a single blood sampling where undetected microfilaraemia may have existed. The lowest prevalence rate of D. immitis infection reported was 12.0 percent (Rajamanickam

et al., 1981). In this study, 233 dogs were examined for circulating microfilariae using the direct smear, the modified Knott's concentration technique and the millipore filter method ('Difil' test-kit, EVSCO Pharma, U.S.A.). The dogs studied were pet dogs from various veterinary clinics in Kuala Lumpur, Petaling Jaya and Klang.

HOST RISK FACTORS

In a study by Kan et al. (1977), the prevalence rates of D. immitis infection among pedigree and non-pedigree dogs were compared. Of a total of 370 dogs, pedigree dogs had a significantly higher prevalence rate (39.2 percent) than the mongrels (27.4 percent). Among the pedigree dogs, the Boxers had the highest incidence of infection followed by the Dobermans. It was also apparent from this study that short haired dogs were more prone to infection than the long haired breeds. This was similarly reported by Stewart et al. (1979). Kan et al. (1977) also noted that there was no significant difference in the distribution of infection between males and females. This is in agreement with the findings of Glickman et al. (1984) and Martin and Collins (1985). Selby et al.(1980) noted a higher prevalence of heartworm infection in hunting and sporting dogs. He attributed this finding to the dogs being more exposed to the infected mosquitoes during hunting and training. Selby et al.(1980) also found that larger dogs (body weight >22 kg) had a higher

prevalence compared to smaller dogs (body weight <14 kg) and stressed that the breed of the dog should always be taken into account. However, contradictory findings were reported by Martin and Collins (1985) who reported that there was no correlation between the body weight of the dog and the prevalence of the dirofilariasis. It was summarised by Selby et al. (1980) that the intact, large male hound, four to seven years of age, has the highest risk of being infected while dogs of smaller breeds, less than two years old, spayed females and castrated males have the lowest risk of being infected. Stewart et al. (1979) screened a total of 81 dogs in North Texas and reported a prevalence of 7.4 percent. Adult heartworms were recovered from a significantly higher percentage of male dogs than females and the male dogs harboured significantly greater number of adult worms than female dogs. Similar findings of the prevalence of infection being higher in males was reported by Faridah and Lee (1981).

The prevalence of infection with D. immitis was reported to increase with the age of the host (Stewart et al., 1979). This is probably because, the older the dog, the greater the chances of it being bitten by infected mosquitoes. This finding was also reported by Faridah and Lee (1981) who noted a significantly higher incidence among older dogs (>4 years).

In a study conducted in Australia, 331 greyhounds were screened for D. immitis using the filtration technique (Martin and Collins, 1985). The results of this study showed